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(54) Title: SOFT TISSUE CLOSURE SYSTEMS

(57) Abstract

A soft tissue closure system (10) for closing percutaneous puncture sites or soft tissue voids, its method of manufacture and method of use are disclosed. The system (10) comprises a delivery means (12) and a self-expandable, resorbable implant (13) disposed within the delivery means (12) in a compressed configuration. Upon release of the implant member (13) from the system (10) within a soft tissue void, the implant member (13) self-expands to conform to the shape of the soft tissue void and seals the void.

SOFT TISSUE CLOSURE SYSTEMS

Related Application

This application is a continuation-in-part of U.S., Patent Application Serial No. 07/881,213, filed May 11, 1992, the contents of which are incorporated herein by reference.

Brief Summary of the Invention

Technical Field

This invention relates generally to the closure of soft tissue sites with self-expandable, bioresorbable, polymer implants, particularly to the closure of percutaneous puncture sites. The present invention is also directed to the delivery of such implants with tubular delivery devices which penetrate the soft tissue sites to a defined depth for hemostasis and wound closure. Methods of preparing the implants are also disclosed.

Background of the Invention

It has been routine practice to insert a catheter through a puncture site into a blood vessel to either treat a diseased blood vessel by a procedure, known in the art as percutaneous transluminal angioplasty (PTA), or to deliver systemic drugs to the blood stream for chemotherapeutic applications. In the case of a PTA procedure, an introducer sheath is inserted into an artery through the puncture site such that a balloon or other type of catheter can then be inserted into the vessel to carry out the procedure within a vessel. Depending upon the nature of the disease and the site of arterial insertion, the size of an introducer sheath can vary from 1 mm to as large a 5 mm. One of the complications of these procedures is hemorrhaging at the percutaneous puncture site after removal of the catheter and the introducer sheath. In order to stop the bleeding, pressure is applied at the puncture site until hemostasis occurs. Since the angioplasty and related procedures often require the use of

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an anticoagulant, the pressure approach is not always effective and
may require a long period of pressurization and occasional surgical
treatment and hospital stay.

A variety of commercial hemostatic products are
available such as those disclosed in U.S. Patent No. 2,465,357; U.S.
Patent No. 3,742,955; and U.S. Patent No. 3,364,200. A felt or fleece
like collagen hemostat is disclosed in U.S. Patent No. 4,066,083. A
hemostatic collagen paste comprising a mixture of collagen powder
and saline is disclosed in U.S. Patent No. 4,891,359. A number of
other collagen based hemostatic materials are disclosed in U.S.
Patents 4,412,947; 4,578,067; 4,515,637; 4,271,070; 4,891,359; 4,066,083;
4,016,877 and 4,215,200. None of these patents teaches the art of
hemostasis at a vessel puncture site.

The use of a collagen based device to close an arterial
puncture site of a blood vessel is disclosed in Ernst, S., Tjonjoegin, R,
Schrader, R, et al. Immediate Sealing of Arterial Puncture Sites
After Cardiac Catheterization and Coronary Angioplasty Using a
Biodegradable Collagen Plug: Results of an International Registry, J.
Am. Coll. Cardiology, 15:851-855, 1993). In order to deliver the
collagen material into the percutaneous puncture site, the puncture
site must be significantly dilated with an oversized applicator sheath.
The delivery cartridge is then inserted through the expanded
puncture site to deliver the collagen material. The collagen is
delivered twice in order to fill the void and secure the hemostasis.
This elaborate procedure with additional wound site expansion has
resulted in significant cases of hematoma in the patients treated with
this device.

Another device to close an arterial puncture site of a
blood vessel is disclosed in U.S. Patent No. 4,744,364 to Kensey. This
device involves the insertion of an expandable, resorbable material
inside the lumen of a blood vessel via a tubular member which fits
inside an introducer sheath. A retraction filament is secured to the
resorbable material for pulling it to the puncture site so that the

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resorbable material engages the inner surface of the blood vessel contiguous with the puncture. The filament is held taut and taped or otherwise secured to patients' skin to hold the resorbable material in position.

The Kensey device introduces several potential risks to the patient. The device may induce an acute thrombosis due to imperfect alignment of the sealing material or to non-hemocompatibility of the material. The premature degradation of the filament may leave the sealing material unsecured, leading to embolization distal to the puncture site. The migration of the sealing material may not only cause rebleeding, but potential thrombosis which requires surgical intervention. The potential risks involved in such a device may outweigh the benefits such a device can offer. Thus, a safe, effective and user friendly method to close a puncture site and stop the bleeding is still highly desirable and welcome.

It has become apparent to Applicant that the key element in solving puncture site closure complications lies in the design of a resorbable matrix having unique characteristics and properties for such an application.

Accordingly, it is the primary object of the present invention to provide a device which will close a puncture site and stop the bleeding while substantially reducing the disadvantages and risks associated with the prior art.

It is a further object of the present invention to provide an implant which self-expands in vivo to fill the voids or defects of a tissue or organ with a biocompatible, resorbable material.

It is still a further object of the present invention to provide a method to deliver the biocompatible, resorbable implant material to tissues or organs of interest by a tubular delivery device.

It is another object of the present invention to provide a means to deliver medicaments, antibiotics, growth factors and other biologically active molecules to selected tissues or organs.

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Its yet another object of the present invention to provide a method of manufacturing the implant.

Summary of the Invention

By means of the present invention, a self expandable, resorbable, hemostatic implant has been discovered which eliminates or substantially reduces many of the disadvantages and problems associated with the prior art attempts at closing punctured wound sites in vessel catheterization and other soft tissue repair procedures. In addition, by means of the present invention, a method is provided to deliver medicaments to the selected site of a soft tissue. More specifically, by means of the present invention, a resorbable, self-expandable, hemostatic implant is delivered to the specific vessel puncture site to stop the bleeding in post angioplasty and related procedures.

The resorbable, self-expandable tissue closure implant of the present invention is generally a dry, compressed porous matrix comprised of biological fibers. As used herein "biological fibers" include collagen, elastin, fibrin and polysaccharides. In a preferred form of the invention, the matrix is comprised of collagen fibers of animal or humans.

In particular, the implant of the present invention comprises a compressed matrix having a density from about 0.10 g/cm^3 to about 1.30 g/cm^3 and pores having an average diameter of from about 0.5 μm to about 50 μm. This compressed matrix self expands when in contact with an aqueous medium resulting in pores with an average diameter of from about 100 μm to about 3,000 μm in its fully expanded configuration, and a corresponding expansion of volume of from about 3 cm^3/cm^3 to about 100 cm^3/cm^3; and a reduction of density of from about 0.10 to about 1.30 g/cm^3 in the compressed configuration to a density of from about 0.01 to about 0.50 g/cm^3 in the fully expanded configuration.

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The matrix may also include selected medicaments for local therapeutic applications. Therapeutic medicaments include, but are not limited to, hemostatic agents such as thrombin, Ca^{++} and the like, wound healing agents such as epidermal growth factor (EGF), acidic and basic fibroblast growth factors (FGFs), transforming growth factors alpha and beta (TGF alpha and beta) and the like, glycoproteins such as laminin, fibronectin and the like, various types of collagens.

The method for fabricating the resorbable, self-expandable soft tissue closure implant, in its broadest embodiment, comprises:

a)   forming an aqueous dispersion containing biological fibers;

b)   pouring the aqueous dispersion into molds;

c)   freeze-drying the aqueous dispersion to form a collagen matrix;

d)   crosslinking the freeze-dried matrix by treatment with crosslinking agent;

e)   spraying the crosslinked matrix with water mist; and then

f)   compressing the water mist treated matrix.

Still further, the invention includes a method for closing a soft tissue puncture site with the resorbable, self-expandable implant. The method comprises delivering the resorbable, self-expandable polymer implant in its compressed configuration to the selected site by a delivery means, particularly a tubular delivery device, and releasing the resorbable implant at the selected soft tissue site where the resorbable implant self-expands to conform to the soft tissue site to close the defect. In particular, the method comprises:

a)   inserting a delivery means having a longitudinal axis and having an outlet at its distal end into the void of the soft tissue to a depth controlled by a depth insertion guide provided on said delivery means;

b)   ejecting an implant, having a length, from said delivery means into the void a controlled distance and in a controlled position, said implant formed of a material capable of being resorbed in the living being characterized by being in a compressed configuration having an average pore size of from about 0.5 μm to about 50 μm, and being self expandable when wetted to have an average pore size of from about 100 μm to about 3,000 μm in the expanded configurtion, wherein (i) the depth insertion guide, (ii) the length of the implant, and (iii) the ejection step, in combination, control the positioning of the implant within the void; and then

c)   removing the delivery means to allow the implant to self-expand and form a hydrated matrix conforming to and sealing the soft tissue void.

The invention also includes a device for sealing a void in a soft tissue of a living being comprising:

a)   an implant, having a length, formed of a material capable of being resorbed in the living being characterized by being in a compressed configuration having an average pore size of from about 0.5 μm to about 50 μm, and self-expandable when wetted having an average pore size of from about 100 μm to about 3,000 μm in the expanded configuration;

b)   a delivery means having an outlet at its distal end and a depth insertion guide, said delivery means being adapted to be inserted into the void to a depth which is controlled by the depth insertion guide, said implant being disposed within said delivery means in a compressed configuration; and

c)   a retractable ejection means capable of ejecting the compressed implant out of the said outlet a controlled distance into the void of the soft tissue to form an expanded, hydrated matrix which conforms to seal the soft tissue void, wherein the (i) the depth insertion guide, (ii) the length of the implant, and (iii) the retractable ejection means, in combination, control the positioning of the implant within the void.

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The resorbable, self-expandable, soft tissue closure implant of the present invention is constructed such that the matrix is highly compressed to provide maximal volume expansion capacity and surface area for fluid absorption, platelet adhesion and hemostasis while maintaining minimal volume for insertion. The highly porous matrix upon expansion also provides maximal surface area for cell infiltration and adhesion for wound healing. Thus, in a preferred embodiment of the present invention, the self-expandable implant of the present invention has the following physical characteristics and physico-chemical properties. It is to be understtod that the properties and characteristics given for the self-expandable implant in the "expanded" state are generally the same as and are also descriptive of the collagen matrix prior to being compressed during the formation of the implant.

Physical Characteristics:

Diameter of the cylinder (cm)			
Compressed		generally:	0.1 - 0.6
		preferred:	0.2 - 0.4
Expanded		generally:	1.0 - 10.0
		preferred:	3.0 - 7.0
Height of the cylinder (cm)		generally:	0.2 - 10.0
		preferred:	0.5 - 5.0
Pore size (μm)			
Compressed		generally:	0.5 - 50
		preferred:	2.0 - 45.0
	most preferred:		5.0 - 40.0
Expanded		generally:	100 - 3,000
		preferred:	200 - 1,000
	most preferred:		250 - 700

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Density (g/cm^3)

Compressed	generally:	0.1 - 1.30
	preferred:	0.2 - 1.0
	most preferred:	0.3 - 0.8
Expanded	generally:	0.01 - 0.50
	preferred:	0.02 - 0.25
	most preferred:	0.03 - 0.15

Physico-chemical Properties:

Swelling Capacity (cm^3/cm^3)	generally:	3 - 100
	preferred:	10 - 50
	most preferred:	15 - 35
Thermal Shrinkage (°C)	generally:	50 - 75
	preferred:	55 - 65
Relaxation Recovery Time (seconds)	generally:	1 - 60
	preferred:	3 - 40
	most preferred:	5 - 30

The invention will next be described in connection with certain illustrated embodiments.

Brief Description of the Figures

Figure 1 is the longitudinal cross-sectional view of one embodiment of the present invention showing a soft tissue closure system comprising an implant delivery means and an implant positioned therein.

Figure 2 depicts the use of the system at a percutaneous puncture site delivering the resorbable implant in cross-sectional view.

Figure 3 depicts the resorbable implant in place in cross-section.

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Detailed Description of the Invention

Referring now to the figures wherein like reference characters refer to the same elements, Figure 1 depicts a soft tissue closure system shown generally as 10 comprising an implant delivery means 12 and an implant member 13 disposed within delivery means 12. The primary function of delivery means 12 is to deliver implant member 13 at a desired site in situ to effect the filling and closure of a void in the soft tissue of a living being. In a preferred embodiment, the soft tissue closure system of the present invention is utilized to effect the closure of a puncture or other opening in a blood vessel, duct or lumen. However, closure system 10 may also be utilized for the treatment of wounds resulting from other voids created to soft tissue in a living being, typically surgeries. Such applications include the filling of voids after removal of malignant tumors, necrotic tissues, degenerative tissues, deep bullet wounds, knife stabbing, and the like. Voids created by plastic or cosmetic surgery, and the like, are also able to be filled using the closure system of the present invention. Still further, the closure system of the present invention may also be used to stop the bleeding and fill the voids in diagnostic applications, such as tissue biopsy where a biopsy needle or other biopsy device is utilized.

While the following description and the Figures are directed to the closure of percutaneous punctures in arteries, it should be understood from the above discussion that the present invention has greater applicability than this preferred embodiment.

Delivery means 12 is made from any biocompatible material, such as, stainless steel; synthetic polymeric materials such as polyethylene, polypropylene, polyvinyl chloride, polystyrene, polytetrafluoroethylene, polyurethane, or the like; natural polymers such as collagen, elastin, or the like; and other such biocompatible materials which are well known to those skilled in the art. Desirably, the delivery means is made from inexpensive, disposable

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materials, such that it is simply discarded after use. For ease of
manufacture and disposability, synthetic polymers are preferred.

      The delivery means shown in the figures comprises a
tubular member 11 having an outlet 20 at its distal end and an ejector
means 22 slidably mounted at the proximal end 28 of tubular member
11. The tubular member is an elongated body having a depth
insertion guide 15, an insertable front portion 32, and flanged
projection 30.

      Insertable front portion 32 has a fixed length "y" which
is selected dependent upon the particular soft tissue site that is being
repaired and will generally vary from about 0.2 cm to about 10 cm. It
is only this insertable front portion 32 of tubular member 11 which
actually is inserted into the patient's skin or the introducer sheath.
The depth of insertion is easily controlled by the depth insertion guide
15 which is positioned on tubular member 11 at the length "y" such
that the tubular member cannot be inserted past this guide. Guide 15
may simply comprise a partial or full circumferential projection or
ledge which will cause the insertion of the delivery means to stop
once the insertable front portion has been inserted, said ledge then
simply resting upon the outermost surface of the patient's skin (or on
the ledge of an introducer sheath, not shown, the use of which is well
known to those skilled in the art). The shape or design of the guide is
not critical as long as it is capable of controlling the depth of the
insertion.

      The insertable front portion 32 is preferably constructed
of an outside diameter which is less than the introducer sheath that
is used for a particular intraluminal procedure so as to enable the
insertable front portion 32 to be easily inserted through the skin (or
the introducer sheath) and be juxtaposed to the percutaneous
puncture site. Depending upon the particular intraluminal
procedure, the outside diameter may vary from about 1 mm to about 6
mm. Except for the insertable front portion 32, the rest of tubular
member 11 may have any outside diameter inasmuch as it does not

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enter the repair site. Desirably, however, for each of construction,
the entire tubular member is typically made having the same inside
and outside diameters.

Flanged projection 30 is arranged to be grasped by the
fingers of the user as the implant member 13 is ejected by ejector
means 22. The flanged projection 30 may completely or partially
circumscribe tubular body 11 at its proximal end 28.

The ejector means 22 comprises an elongated,
cylindrical rod-like member 24 having a push plate 14 attached to its
distal end being in a plane which is perpendicular to its longitudinal
axis and a thumb rest 26 attached to its proximal end also being in a
plane which is perpendicular to the longitudinal axis of rod-like
member 24. Push plate 14 is disposed inside of tubular member 11, in
insertable portion 32, and has an outside diameter which is slightly
less than the inside diameter of insertable portion 32 to enable the
push plate 14 to travel down the longitudinal axis of insertable
portion 32, to push or force implant member 13 out of the outlet 20.
Rod-like member 24 is able to be retracted to a distance of "d", as
shown in Figure 1, which distance is only slightly longer than the
length of the implant member 13, so as to ensure complete release of
the implant and, most importantly, not push the implant a distance
beyond that which is desired. Thus by controlling the length "y" of
insertable portion 32, the length of the implant member 13, and
distance "d", the implant member 13 is precisely positioned at the
repair site.

In use, the closure system of the present invention is
desirably utilized as soon as possible after the removal of the
introducer sheath or while the introducer sheath is partially
removed from the puncture site. As shown in Figure 2, insertable
portion 32 is inserted at the repair site through the puncture 42 in
skin 40. Depth insertion guide 15 controls the depth of insertion,
stopping the travel of the insertable portion 32 once the guide rests
upon skin 40. To effect the delivery of the implant member to the

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repair site, the user grasps projection 30 with his fingers and places his thumb on rest 26. By applying pressure to the thumb-rest, the ejector means travels a distance "d" thereby ejecting implant member 13 and delivering it directly over the percutaneous puncture site 42 of vessel 44.

As more clearly shown in Figure 3, implant member 13 is not positioned inside of the lumen vessel 44, but rather, by the controlled insertion depth provided by the present invention, is delivered extravascularly such that end 46 of the implant member rests outside and directly on top of the puncture vessel. The ejected implant member 13 expands quickly in situ to completely close the puncture site as the delivery device is slowly being removed.

Figure 3 is a schematic representation of a closed puncture site. The implant member 13 is shown as having considerably swelled and the blood around the implant member has been absorbed and·clotted through a collagen induced hemostatic mechanism forming blood clot 50. A wound bandage is shown as 52.

The implantable, resorbable member 13 is made primarily from biopolymers, such as proteins, polysaccharides or the like. Preferably, a collagen based material is used due to its intrinsic hemostatic properties.

Type I to type XIV collagens may be used either singularly or in combination for the manufacture of the implantable member 13. Preferably, type I collagen is used due to the availability of this material in large quantity, the ease of its isolation and purification, and proven hemostatic properties. The primary source of type I collagen is tendon, skin, bone, and ligament. Both human and animal tissues may be used to isolate the collagen. In general, animal tissues are preferred due to easy availability in fresh forms from local slaughter houses.

In preparing the implantable member 13, type I collagen is first isolated and purified. A review of the preparation of collagen can be found in "Methods in Enzymology," vol. 82, pp. 33-64, 1982. In

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particular the collagen of the present invention may be prepared by
the following method.

First, a native source of type I collagen, such as skin,
tendon, ligament or bone is first cleaned of fat, fascia and other
extraneous matter and washed. The clean and washed collagen
containing material is then comminuted by slicing or grinding. Bone
material is subsequently subjected to a demineralization procedure.
This is achieved either with an acid solution such as hydrochloric
acid or a solution of chelating agent such as
ethylenediaminetetraacetic acid (EDTA).

The material is then subjected to a defatting treatment
using fat solubilizing agents such as ethanol, propanols, ethers or a
mixture of ether and alcohol. The defatted collagen containing
material is then extracted in a neutral salt solution to remove neutral
salt soluble material. Typically, 1 M NaCl solution is used for this
purpose. The high ionic strength salt solution weakens the non-
specifically bound non-collagenous materials which are solubilized
and removed. The salt extracted collagen containing material is
then washed with deionized, distilled water.

The neutral salt extracted collagen containing material
is then subjected to an acid extraction in the presence of a structure
stabilizing salt to further remove acid soluble non-collagenous
materials. Applicable acids include acetic acid, lactic acid,
hydrochloric acid, sulfuric acid, phosphoric acid, and the like.
Regardless of which acid is used, the pH of the acid solution is
adjusted to be below 3. The salt used include sodium chloride,
ammonium sulfate, sodium sulfate, or the like. Acid extraction
weakens the interaction between the collagen and the acidic non-
collagenous impurities which are solubilized and removed.

The acid extracted collagen is then neutralized by
adjusting the pH to its isoelectric point at pH of from about 6 to about 7
by adding a base. Applicable bases include sodium hydroxide,
potassium hydroxide, ammonium hydroxide, and the like. By

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adding a base, the collagen coacervates. The coacervated collagen is then filtered by means well known in the art such as using·a stainless steel mesh filter under vacuum.

The acid extracted, base neutralized collagen is then washed with deionized, distilled water to remove the residual salt formed by the neutralization procedure. The washed collagen is then subjected to a base extraction in the presence of a structure stabilizing salt. Such bases are well known in the art such as sodium hydroxide, potassium hydroxide, calcium hydroxide and the like. Regardless of which base is used, the pH of the solution is adjusted to be above 13. Base extraction weakens the interaction between collagen and the basic non-collagenous impurities. By adding the base, non-collagenous materials are solubilized and removed. The base also lowers the isoelectric point due to a partial deamidation of glutamines and asparagines in collagen which produce additional carboxyl groups. The base extracted collagen is then coacervated by adjusting the pH to its isoelectric point at a pH of from about 4.5 to 5.5 by adding an acid to the collagen dispersion so as to fully separate the fibers from the solution for ease of filtration. Such acids include hydrochloric acid, sulfuric acid, acetic acid, lactic acid, phosphoric acid and the like. The coacervated collagen is then filtered. After discarding the extraction solution, the fibers are washed with deionized, distilled water to remove the residual salts resulting from neutralization of the extraction solutions. The thusly purified collagen is stored in a freezer or stored in freeze-dried form for the preparation of collagen implantable member 13.

To fabricate a collagen implantable member 13, a collagen dispersion is first prepared in a manner well known in the art. One such preparation is taught in U.S. Patent No. 3,157,524, which is incorporated herein by reference as if set out in full. Another preparation of collagen dispersion is taught in U.S. Patent No. 3,520,402 which is also incorporated as if set out in full.

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In particular, the collagen dispersion of the present invention may be prepared by the following method.

The purified collagen material is first dispersed in 1×10^{-4} M NaOH solution to swell the collagen fibers. The collag material is then homogenized by any conventional means such as with a blender or homogenizer so as to fully disperse the fibers. The homogenized collagen is then filtered to remove any unswollen aggregates by means well known in the art such as by passing the dispersion through a stainless steel mesh screen. The pH of the dispersion is adjusted to about 7.4 by adding 0.01 M HCl. The initial dispersion in a base allows the neutralization step to be carried out without passing the isoelectric point so as to not cause coacervation of the collagen and obtain a more uniform dispersion at pH 7.4. The dispersion is then de-aired by vacuum. The resulting collagen dispersion may then be used to prepare the self-expandable, implantable soft tissue closure device.

Typically, the weight percent of collagen in a dispersion is from about 0.5 to about 5.0, preferably in the range from about 0.75 to about 2.0. Higher weight percent of collagen than 5.0 in the dispersion can be obtained by centrifuging the dispersion in a centrifuge and discard the supernatant. Generally, the higher the centrifugal force applied to the dispersion, the higher the weight percent of collagen in the dispersion after removing the supernatant.

In one embodiment of the present invention, if the collagen soft tissue closure system is intended to function as a medicinal delivery vehicle, then in addition to the type I collagen, medicinal additives may optionally be included in the dispersion, such as antibiotics, thrombin, polysaccharides such as hyaluronic acid, chondroitin sulfates, alginic acids, chitosan and the like, growth factors such as epidermal growth factors, transforming growth factor beta (TGF-B) and the like, glycoproteins such as fibronectin, laminin, and the like, type II through type XIV collagens, and mixtures thereof.

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The collagen dispersion is then poured into molds. The shape of the mold may be cylindrical, rectangular, spherical or any other shape so long as the size of the mold is larger than the inner diameter of insertable front portion 32. For a 2 mm internal diameter (I.D.) insertable front portion 32, and a cylindrical implantable collagen member 13, the diameter of the mold is preferably in the range of from about 3 mm to about 15 mm and the height of the mold is from about 5 mm to about 25 mm.

The molds containing the dispersion are then placed in a freezer maintained at a temperature of from about -10°C to about -50°C for a length of time sufficient to freeze the water present in the dispersion, generally for a about 1 to about 24 hours. The frozen dispersion is then subjected to freeze-drying so as to remove the frozen water. This freeze-drying procedure is carried out in a commercial freeze dryer, such as that manufactured by Virtis, Stokes or Hull, at conditions well known to those skilled in the art. Typically, the vacuum within the drying chamber is maintained at from about 50 μm to about 300 μm of Hg, at a temperature of from about -10°C to about -50°C for about 16 to about 96 hours. The temperature is then raised to about 25°C for about 3 to 24 hours.

The freeze-dried, highly porous collagen matrix is then subjected to a crosslinking process to introduce additional intermolecular crosslinks to stabilize the form of the collagen matrix. The crosslinking is carried out by means well known in the art. Any reagents which can chemically react with amino groups, hydroxyl groups, guanidino groups, carboxyl groups that can link the side chains of different collagen molecules may be used to crosslink the collagen matrix. This can be accomplished with chromium sulfate, formaldehyde, glutaraldehyde, carbodiimide, adipyl chloride, hexamethylene diisocyanate and the like. The rate of in vivo resorption of the collagen is dependent upon the degree of intermolecular crosslinking in the collagen matrix. Factors controlling the extent of crosslinking are the type and concentration

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of the crosslinking agent; the pH, time and the temperature of
incubation in the liquid phase; or the vapor pressure of the ·
crosslinking agent, time, temperature and the relative humidity
when carrying out crosslinking in the vapor phase. Desirably, the
collagen matrix of the present invention is crosslinked to the extent
that the collagen is completely resorbed within about 2 to about 10
weeks.

Appropriate crosslinking of the freeze-dried matrix
introduces several very important properties of the present invention
for the specified medical applications as a soft tissue closure device.
Effective crosslinking locks in the physical geometry of the matrix
which is defined by the shape of the mold. Consequently, the matrix
behaves elastically when a stress is applied to the matrix. That is,
when the matrix is deformed or compressed physically, it will return
to its original form and size upon relaxation or release of the external
stress, or expand to an extent which conforms to a physical barrier,
such as the walls of the puncture site. This elastic behavior of the
appropriately crosslinked freeze-dried collagen matrix is especially
manifested when the collagen matrix is in the wet state, as when it
absorbs blood at the puncture site. This is a result of hydrophilicity
and the Donnan osmotic pressure of the collagen matrix. The
recovery time from the deformed or compressed state to the original
shape in the wet state is as short as from about 1 second to about 60
seconds. Desirably, the recovery time is from about 3 to about 30
seconds.

Another important property caused by crosslinking the
collagen matrix is its volume expansion capacity from the
compressed state to the expanded state. The volume expansion
capacity of the crosslinked collagen matrix of the present invention is
limited by the size of the mold which defines the total volume of the
matrix of the present invention. The total volume of a matrix is
defined by the geometry of the mold. Typically, when the mold is of
the form of a cylinder, the volume is defined by the area of the base of

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the cylinder and the height of the cylinder. The desirable volume of
the matrix when in use for puncture site closure is dependent upon
the particular size of the introducer sheath used. Particularly, when
a 9F (about 3 mm) introducer sheath is used, the desirable dimension
of a cylindrical matrix is to have a base dimension of from about 6
mm to about 15 mm, and a height of from about 5 mm to about 25
mm. This means that the volume of the compressed matrix will
expand about 3 to 20 times upon delivery and self expansion.

     Yet an additional property controlled by crosslinking the
collagen matrix is its density. Dependent upon the particular
puncture site to be repaired and the physical, chemical and biological
requirements, the density of the compressed matrix is in the range of ·
from about 0.1 g/cm^3 to about 1.30 g/cm^3, and the density of the
matrix in the fully expanded configuration may vary from about 0.01
g/cm^3 to about 0.5 g/cm^3. Typically, to close a puncture site from
angioplasty or related procedures, the density of the matrix in the
compressed state various from about 0.25 g/cm^3 to about 1.0 g/cm^3,
and in the fully expanded configuration various from about 0.02
g/cm^3 to about 0.15 g/cm^3.

     Another property of the crosslinked collagen matrix is
its pore structure. The fully expanded crosslinked collagen matrix
has an average pore size from about 100 μm to about 3,000 μm. The
average pore size is drastically reduced in the compressed state to a
value of from about 0.5 μm to about 50 μm. The compression of the
crosslinked matrix permits the matrix to be inserted into a small
volume for delivery and the subsequent self expansion of the matrix
upon delivery allows the matrix to expand to the extent necessary to
effectively conform to the puncture hole and seal it. Of course, the
implant comprised of the compressed crosslinked collagen matrix
material is chosen having particular physical dimensions keeping in
mind the size of the puncture site which is to be closed such that
when the implant self-expands, it expands to such an extent and size

that it is capable of exerting pressure against the walls of the puncture site thereby conforming to it and effectively closing the site.

The degree of crosslinking of the collagen matrix of the present invention can be measured by the hydrothermal shrinkage temperature (T_S) of the matrix, i.e. the onset temperature at which the matrix begins to shrink in its dimension in an aqueous environment as a result of the unwinding of the triple helical structure of the collagen molecules. The methods for measuring the shrinkage temperature of a material is well known in the art, such as by a differential scanning calorimeter, or by measuring the dimensional change using a cathetometer.

Generally, the degree of crosslinking is such that the shrinkage temperature of the collagen matrix is in the range of from about 50°C to about 75°C, preferably from about 55°C to about 65°C.

In one embodiment of the present invention, the collagen matrix is crosslinked with formaldehyde vapor. Either commercial formaldehyde vapor, or vapor of formaldehyde generated from a formaldehyde solution may be used. Particularly, the crosslinking is conducted in a chamber with a relative humidity in the range of from about 80% to about 100%, preferably in the range from about 85% to about 95%, and in the presence of an excess amount of formaldehyde vapor, at a temperature of about 25°C for a period from about 30 minutes to about 8 hours. Specifically, crosslinking by formaldehyde vapor generated from 1% formaldehyde solution at 25°C and at 95% humidity for 60 minutes produce a collagen matrix of a shrinkage temperature of from about 55°C to about 65°C for a matrix of density from about 0.02g/cm^3 to about 0.15 g/cm^3 in the fully expanded configuration.

The crosslinked collagen matrix is then subjected to a water mist treatment. Any commercial water mist sprayer is suitable for this purpose. The collagen matrices are sprayed for about 10 seconds to about 60 seconds while the collagen matrices are being tumbled in a container at about 25°C. The water mist treated

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matrices are then equilibrated in a closed container for about 30 minutes to further soften the matrices for the compression step which follows. As a result of this water treatment, the collagen matrices have a water uptake of about 10 to 40 % by weight, based on the weight of the dry material. The water mist treated collagen matrix is then subjected to mechanical compression to reduce its size in order to fit into insertable portion 32. Particularly, when the matrix is in a cylindrical form, the mechanical compression is applied in the radial direction such that the base area is reduced to approximately the size of the I.D. of insertable portion 32. Generally, the compressed collagen matrix has a volume of from about 1/100 to 1/3 of the non-compressed matrix. The compressed collagen implantable member 13 is then inserted into insertable front portion 32. At this point, the loaded soft tissue closure system is individually packaged for sterilization.

The crosslinking of the matrix, the water mist treatment and the mechanical compression are important aspects of the present invention. More importantly, the sequence of operation as described in this invention is critical in providing the desirable properties of the collagen matrix. The density, the pore structure and the extent of swelling of the compressed collagen implant member is directly related to how the collagen matrix material was made. For example, a change of the order from the present invention to water mist treatment, mechanical compression and then crosslinking the matrix will result in a matrix which will not self-expand when the stress is released and which does not have the blood absorption capability.

While only the sealing of a percutaneous puncture seal has been discussed as an example to describe the present invention, it will be understood that various changes, modifications, and applications may be made without departing from the scope and the spirit of the present invention.

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The above and other objectives, advantages and features of the present invention will be better understood from the following examples.

Example 1
Preparation of the Collagen Dispersion

The fat and fascia of the bovine flexor tendon are carefully cleaned and removed and washed with water. The cleaned tendon is frozen and diminuted by slicing into 0.5 mm slices with a meat slicer. The tendon is first defatted with isopropanol (tendon : isopropanol = 1:5 vol:vol) for 8 hours at 25°C under constant agitation. The extraction solution is discarded and equal volume of isopropanol is added and the tendon slices is extracted overnight at 25°C under agitation. The tendon is then extensively washed with deionized, distilled water to remove the residual isopropanol. The defatted tendon is then extracted with 10 volumes of 1 M NaCl for 24 hours at 4°C under agitation. The salt extracted tendon is washed with deionized, distilled water. The fibers are next extracted with 10 volumes of 1.0 M NaOH for 24 hours at 25°C in the presence of 1 M Na_2SO_4 under constant agitation. The alkaline extracted collagen is then collected by filtration and neutralized with 0.1 M HCl and the fibers collected, washed to remove the residual salt and frozen.

An aliquot of the above purified fibers is first suspended in 1×10^{-4} M NaOH solution. The amount of fibers and base solution used is such that a 1.5% (wt/vol) of collagen suspension is reached. The swollen fibers are then homogenized in a stainless steel blender for 60 seconds. The thusly dispersed collagen material is filtered through a 40 μm stainless steel mesh. The pH of the dispersion is then adjusted to about 7.4 by adding 0.01 M HCl. The dispersed material is then de-aired by vacuum and stored at 4°C until use.

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Example 2
Preparation of Collagen Soft Tissue Closure Implant

Collagen dispersion prepared from Example 1 is poured into stainless steel molds of 15 mm in diameter and 10 mm in height. The collagen containing molds are then subjected to a freeze-drying procedure using a Virtis commercial freeze dryer. The conditions for freeze-drying are: freeze at -40°C for 6 hours, drying at 150 μm Hg at -10°C for 24 hours followed by drying at 25°C for 8 hours. The freeze-dried collagen matrices are then subjected to a formaldehyde vapor crosslinking in a crosslinking chamber containing excess amount of formaldehyde vapor (generated by a 1% formaldehyde solution at 25°C), 95% relative humidity at 25°C for 60 minutes. The crosslinked collagen matrices are sprayed with water mist for 10 seconds and equilibrated in a closed container for an additional 30 minutes. The water mist treated matrices are then compressed by rotating between two glass plates with a gap of 2.5 mm such that the diameter of the 15 mm sponge matrix reduces to about 2.5 mm. The compressed collagen matrix is then inserted into a pre-fabricated implant delivery means of 2.5 mm I.D. and 3.0 mm O.D. (member 11, Figure 1).

Example 3
Preparation of Collagen Soft Tissue Closure Implant in the Presence of Thrombin

The collagen dispersion from Example 1 is mixed uniformly with thrombin (collagen : thrombin = 10:1 wt/wt). The thoroughly mixed collagen/thrombin gel is then poured into the stainless steel molds as in Example 2. The subsequent steps are identical to the Example 2.

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Example 4
Characterization of Collagen Soft Tissue Closure Implant

a) <u>Density (g/cm^3)</u>

The apparent density of the soft tissue closure implant in the compressed and fully expanded configuration is determined by first weighing the collagen matrix to obtain the dry weight. The volume of the matrix is then determined from the radius and the height of the sponge according to: $V = \pi \times r^2 \times h$, where r is the radius and h is the height of the matrix. The density of the collagen soft tissue closure implant of the present invention is in the range of from about 0.10 g/cm^3 to about 1.30 g/cm^3 for the compressed matrix, and is from about 0.01 g/cm^3 to about 0.5 g/cm^3 for the fully expanded matrix.

b) <u>Swelling (cm^3/cm^3)</u>

The swelling is defined as the expansion of the volume of the matrix per unit volume of the soft tissue closure device. The volume of the compressed collagen matrix is first determined by measuring the dimensions of the matrix. The collagen matrix is next immersed in a buffered solution at pH 7.4 at 25°C for 5 minutes. The volume of the expanded wet matrix is then determined. The swelling (cm^3/cm^3) of the collagen matrix is calculated as the volume of the expanded matrix divided by the volume of the compressed matrix. The swelling capacity of the collagen soft tissue closure implant of the present invention is in the range of from about 3 cm^3/cm^3 to about 100 cm^3/cm^3.

c) <u>Pore Size (μm)</u>

The pore size is obtained from the scanning electron micrographs (SEM) of cross-sections of the collagen implant in its compressed and fully expanded configurations. The pore size of the compressed matrix is defined as the gap distance of a compressed pore. The pore size of the expanded matrix is defined as the average of the longest distance and the shortest distance of an open pore. The

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pore size of the collagen matrix of the present invention is in the range of from about 0.5 μm to about 50 μm for the compressed implant, and is from about 100 μm to about 3,000 μm for the fully expanded implant.

d) Relaxation Recovery Time (seconds)

The collagen soft tissue closure implant of the present invention in its compressed configuration is pushed out from the disposable delivery means into a buffered solution, pH 7.4 at 25°C. The compressed matrix is relaxed, hydrated and self expanded to the fully expanded configuration. The time it takes to recover to the fully expanded configuration is recorded. The relaxation recovery time for the present invention is in the range of from about 1 second to about 60 seconds.

e) Shrinkage Temperature (°C)

A 10 mg sample of the collagen matrix is first wetted in a buffered solution, pH 7.4. The sample is sealed into an aluminum sample pen and inserted into a sample holder of a differential scanning calorimeter. The buffer solution is used as a reference. The heating rate is 5°C/min. The shrinkage temperature is defined as the onset of the endothermic peak from the heat capacity versus temperature plot. The thermal shrinkage temperature of the collagen soft tissue closure implant of the present invention is in the range of from about 50°C to about 75°C.

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Example 5
Method of Use of a Collagen Soft Tissue Closure Implant

An appropriately sized collagen soft tissue closure
implant is inserted into a puncture site with the introducer sheath
partially removed. The collagen implant is ejected into the puncture
site and allowed to be fully hydrated and self-expanded in situ for 5
minutes. The dispersed delivery means is then slowly withdrawn.
Slight pressure is then applied to the wound for 5 to 10 minutes to
ensure complete hemostasis and wound closure.

Example 6
Method of Use to Close a Soft Tissue Site

An appropriately sized delivery means is inserted
through a percutaneous site to the tissue site of interest. The
collagen implant is then pushed out of the tubular delivery means via
a piston to the tissue site while the delivery means is slowly being
withdrawn. The collagen implant self expands to fill the voids of the
soft tissue site.

The present invention may be embodied in other specific
forms without departing from the spirit or essential characteristics
thereof. The present embodiments are therefore to be considered in
all respects as illustrative and not restrictive, the scope of the
invention being indicated by the appended claims rather then by the
foregoing descriptions.

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What is claimed is:

1.   A device for sealing a void in a soft tissue of a living being comprising:

    a)   an implant, having a length, formed of a material capable of being resorbed in the living being characterized by being in a compressed configuration having an average pore size of from about 0.5 μm to about 50 μm, and self-expandable when wetted having an average pore size of from about 100 μm to about 3,000 μm in the expanded configuration;

    b)   a delivery means having an outlet at its distal end and a depth insertion guide, said delivery means being adapted to be inserted into the void to a depth which is controlled by the depth insertion guide, said implant being disposed within said delivery means in a compressed configuration; and

    c)   a retractable ejection means capable of ejecting the compressed implant out of the said outlet a controlled distance into the void of the soft tissue to form an expanded, hydrated matrix which conforms to seal the soft tissue void, wherein the (i) the depth insertion guide, (ii) the length of the implant, and (iii) the retractable ejection means, in combination, control the positioning of the implant within the void.

2.   The device of Claim 1, wherein the self-expandable resorbable implant is formed of a biocompatible, bioresorbable material.

3.   The device of Claim 2, wherein the biocompatible, bioresorbable material is type I collagen.

4.   The device of Claim 1, wherein the compressed implant has a density of from about 0.1 g/cm^3 to about 1.30 g/cm^3, a

swelling capacity of from about 3 cm^3/cm^3 to about 100 cm^3/cm^3, and a relaxation recovery time of from about 1 second to about 60 seconds.

5.   The device of Claim 1, wherein the delivery means is a tubular member having a longitudinal axis and wherein the ejection means comprises a pusher member located within the tubular member and arranged to move down the longitudinal axis to force the implant out of the outlet.

6.   The device of Claim 5, wherein the delivery means is formed of a biocompatible material.

7.   The device of Claim 6, wherein the biocompatible material is a synthetic polymeric material.

8.   The device of Claim 1, wherein the implant self expands to conform to the tissue void when in contact with body fluid.

9.   The device of Claim 1, wherein the implant is of any geometrical shape.

10.   The device of Claim 1, wherein the depth insertion guide comprises a projection extending outwardly from the longitudinal axis of the delivery means, the distance between the projection and the outlet defining the depth of insertion of the delivery means.

11.   The device of Claim 1, wherein the projection rests upon the outermost layer of the living being skin when the delivery means is inserted to the depth of insertion.

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12.   The device of Claim 1, wherein the projection rests upon the ledge of an introducer sheath when the delivery means is inserted to the depth of insertion.

13.   The device of Claim 1, wherein the soft tissue void is an incision or puncture in the wall of a blood vessel extending to the surface of the skin and the depth insertion guides the depth of insertion such that the ejected implant is positioned juxtaposed to the outside wall of said blood vessel at the incision or puncture site.

14.   A method of making a self-expandable, resorbable collagen implant comprising:
a)   forming an aqueous dispersion containing collagen;
b)   pouring the aqueous dispersion into molds;
c)   freeze drying the aqueous dispersion to form a collagen matrix;
d)   crosslinking the collagen matrix by treatment with crosslinking agent;
e)   spraying the crosslinked collagen matrix with water mist; and then
f)   compressing the water mist treated collagen matrix.

15.   The method of Claim 14, wherein the dispersion further contains medicaments including antibiotics, growth factors, thrombin, glycosaminoglycans, prostaglandins, type II through type XIV collagens, glycoproteins, fibronectins, laminin, and mixtures thereof.

16.   The method of Claim 14, wherein the crosslinking agent is formaldehyde.

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17.   The method of Claim 16, wherein the degree of crosslinking is such that the collagen implant resorbs in about 2 weeks to about 10 weeks.

18.   The method of Claim 14, wherein the resorbable collagen implant has a density of from about 0.1 g/cm^3 to about 1.30 g/cm^3 in the compressed configuration, and from about 0.01 g/cm^3 to about 0.5 g/cm^3 in the expanded configuration.

19.   The method of Claim 14, wherein the resorbable collagen implant has a swelling capacity of from about 3 cm^3/cm^3 to about 100 cm^3/cm^3.

20.   The method of Claim 14, wherein the resorbable collagen implant has a relaxation recovery time of from about 1 second to about 60 seconds.

21.   The method of Claim 14, wherein the resorbable collagen implant has a thermal shrinkage temperature of from about 50°C to about 75°C.

22.   A method of sealing a void in soft tissue of a living being comprising:

a)   inserting a delivery means having a longitudinal axis and having an outlet at its distal end into the void of the soft tissue to a depth controlled by a depth insertion guide provided on said delivery means;

b)   ejecting an implant, having a length, from said delivery means into the void a controlled distance and in a controlled position, said implant formed of a material capable of being resorbed in the living being characterized by being in a compressed configuration having an average pore size of from about 0.5 µm to about 50 µm, and being self expandable when wetted to have an

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average pore size of from about 100 μm to about 3,000 μm in the expanded configurtion, wherein (i) the depth insertion guide, (ii) the length of the implant, and (iii) the ejection step, in combination, control the positioning of the implant within the void; and then

      c)   removing the delivery means to allow the implant to self-expand and form a hydrated matrix conforming to and sealing the soft tissue void.

      23.   The method of Claim 22, wherein the soft tissue void is an incision or puncture in the wall of a blood vessel extending to the surface of the skin and the depth insertion guide guides the depth of insertion of the delivery means such that the ejected implant is positioned juxtaposed to the outside wall of said blood vessel at the incision or puncture site.

      24.   The method of Claim 22, wherein the living being has skin which skin has an outermost layer and wherein the depth of insertion is controlled by the depth insertion guide which comprises a projection extending outwardly from the longitudinal axis of the delivery means, defining a distance between the projection and the outlet which further defines the depth of insertion of the delivery means and where the projection rests upon the outermost layer of the skin of the living being when the delivery means is inserted to the depth of insertion.

      25.   The method of Claim 22, wherein the implant is a biocompatible, polymeric material.

      26.   The method of Claim 25, wherein the biocompatible, polymeric material is a collagen containing material.

      27.   The method of Claim 22, wherein the implant self-expands when contacted with body fluid or blood.

28.   The method of Claim 22, wherein the compressed implant has a density of from about 0.10 g/cm^3 to about 1.30 g/cm^3 in the compressed configuration, and from about 0.01 g/cm^3 to about 0.50 g/cm^3 in the expanded configuration; a swelling capacity of from about 3 cm^3/cm^3 to about 100 cm^3/cm^3; a shrinkage temperature of from about 50°C to about 75°C; and a relaxation recovery time of from about 1 second to about 60 seconds.

29.   The method of Claim 22, wherein the compressed implant further contains medicaments including antibiotics, growth factors, thrombin, glycosaminoglycans, prostaglandins, type I through type XIV collagens, glycoproteins, fibronectins, laminin and mixtures thereof.

30.   The self-expandable, resorbable collagen implant prepared by the method of Claim 14.

Fig. 1

Fig. 2
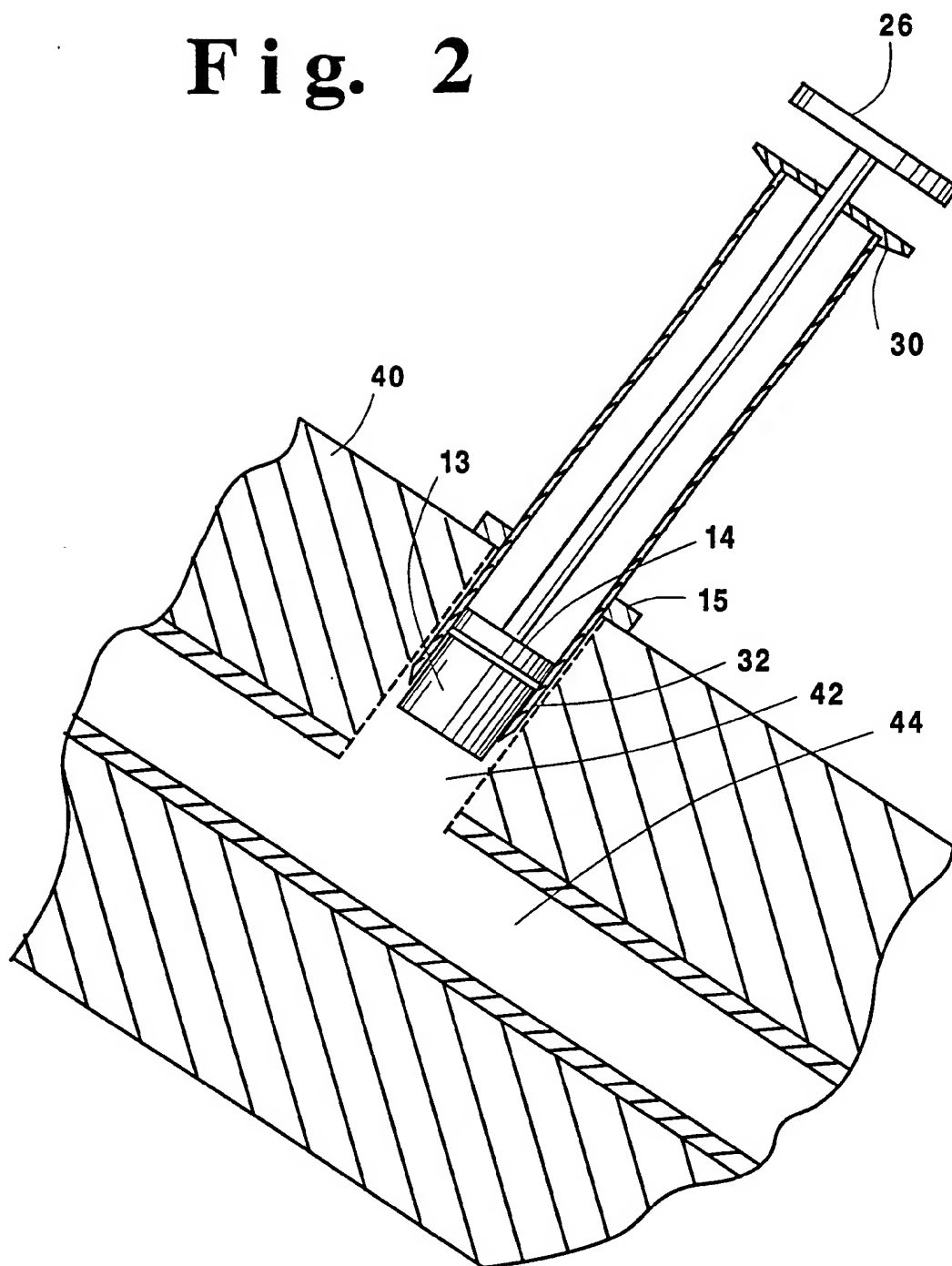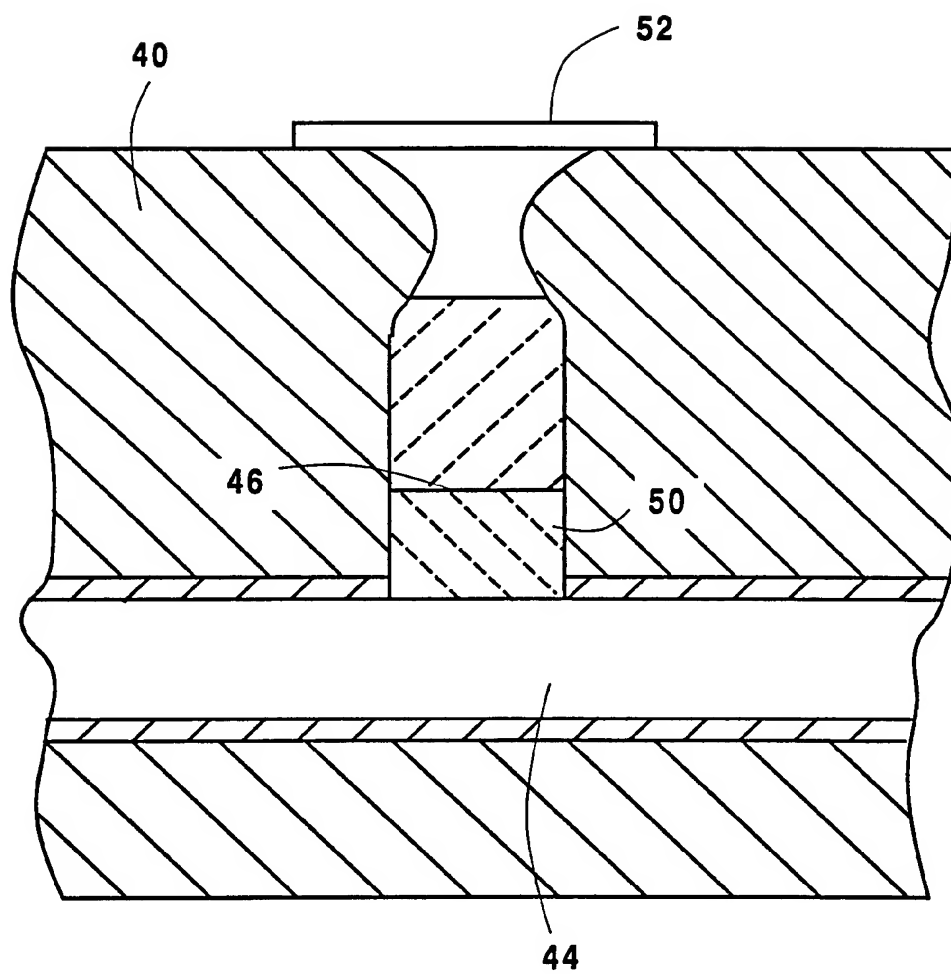
Fig. 3

INTERNATIONAL SEARCH REPORT

Inte .ional application No.

PCT/US94/02973

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :A61F 2/02, 13/20

US CL :128/DIG.8; 604/15, 21, 117, 208, 228; 623/11

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 128/DIG.8; 604/15, 21, 117, 208, 228; 623/11

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

NONE

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

NONE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	FR, A, 7411-839, (SINIGAGLIA L.), 28 November 1975. See the Abstract.	1-13, 22-30
A	US, A, 4,970,298, (SILVER ET AL.), 13 November 1990.	1-30
A	US, A, 5,14,496, (DALTO ET AL.), 25 August 1992.	1-13, 22-30
A	US, A, 4,744,364, (KENSEY), 17 May 1988.	1-30

☐ Further documents are listed in the continuation of Box C.   ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be part of particular relevance		
"E"	earlier document published on or after the international filing date	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
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"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed	"&"	document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
25 JUNE 1994	AUG 02 1994

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